

ANALYSIS OF DIARRHEAL DISEASES IN HUMANS CAUSED BY *SALMONELLA* IN BULGARIA

Maria Pavlova, PhD, mimipavlovaa@gmail.com

| Rositsa Stoyanova

NRL for Enteric Diseases, Pathogenic Cocci and Diphtheria,
National Center for Infectious and Parasitic Diseases, Sofia, Bulgaria

Introduction. The incidence of salmonellosis among people in Bulgaria in recent years has remained relatively high, despite the annual fluctuations. With this work, we aim to clarify the dynamics in the spread of human infections for 2017 -2023 in Bulgaria. **Materials and Methods.** Data from annual analyses of 3208 *Salmonella* cultures from patients and contacts of the NRL "Enteric Infections, Pathogenic Cocci and Diphtheria" and primary microbiological laboratories in the country were used. Also, data on reported cases of salmonellosis from all 28 Regional Health Administrations, data from the National Centre for Public Health and Analysis and the end annual analyses of communicable diseases in Bulgaria prepared by the Epidemiology Department of the National Centre for Infectious and Parasitic.

Results and Discussion. During the study period, a total of 3208 human salmonellosis cases were laboratory confirmed by bacterial culture isolation, of which 1847 were from patients with enterocolitis syndrome and 1361 from infectious carriers. These cultures represent two species of *Salmonella* enterica and *S. bongori*, 4 subtypes- enterica; salame; arizonae and diarizonae and 66 *Salmonella* serotypes. *Salmonella* Enteritidis was the leading etiological agent in the patients, with a relative share of 47%, followed by *Salmonella* Typhimurium- 32.8%, 40.5%- monophasic. The third place is held by *Salmonella* Infantis - 4,85%. The following serotypes were isolated for the first time in our country: Brazzaville; Larochelle; Ndolo; Kuoka; Jerusalem; Vejle and the unique for the world, new serotype- *Salmonella* 11: z41: enz15. In carriers, in contrast to patients, other serotypes come to the fore- Muenster; Bazenheid; Dublin, etc. The data obtained from the analysis again confirm that the group most affected by *Salmonella* infections is the group of infants and young children. *Salmonella* outbreaks in the country have been decreasing dramatically since 2020, in contrast to sporadic cases. In 2018, an outbreak of a serotype rare in the country- London was recorded. **Conclusion.** From the above data, it is evident that *Salmonella* infections continue to be a complex public health problem, making it imperative that in the fight to contain them, their aetiological structure is well understood and monitored dynamically.

Keywords: *Salmonella* sp.; salmonellosis; enterocolitis; outbreak.

Acute infectious diarrhoeal disease or the so-called "diarrhoeal syndrome" remains a pressing issue globally for developing countries and a different, but significant, problem for developed countries. In several developing countries, over 25% of all paediatric patients have infectious diarrhoeal syndrome, and of these, over 80% are under 2 years of age (*GBD 2016 Diarrhoeal Disease Collaborators*). The second most affected age group is patients over 70 years of age, and in not a few cases the infectious process is lethal (*Christopher Troeger, et. al., 2019*). Salmonellosis is the second, after campylobacteriosis, most commonly reported gastrointestinal infection and an important cause of both sporadic cases and foodborne outbreaks in the European Union and the European Economic Area (ECDC) (*ECDC; 2022*). A report from the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control's Zoonoses Monitoring and Surveillance reports 15.3 per 100,000 salmonellosis cases for 2022. The CDC estimates that approximately 1.35 million illnesses and 420 deaths are caused by non-typhoidal *Salmonella* annually in the United States (*CDC; 2019*). In Bulgaria, the incidence of human salmonellosis has remained relatively high in recent years despite year-to-year fluctuations. In a 2006 study, Aseva et al. reported increased resistance among the leading serotypes causing human salmonellosis in our country (*Asseva G., et. al., 2006*). The authors highlighted Corvallis as a rare serotype in Europe, but in Bulgaria, it has been the third most frequently reported causative agent of human infection after *S. Enteritidis* and *S. Typhimurium* since 1997 (*Asseva G., 2006*). With the present work, we aim to clarify the dynamics in the prevalence of *Salmonella* causing human infections for the period 2017-2023 as an important health and socio-economic problem.

Materials and Methods. To perform the retrospective epidemiological study, data from the annual analyses of isolated 3208 *Salmonella* cultures from patients and contacts of the NRL Enteric Infections, Pathogenic Cocci and Diphtheria, as well as strains obtained for confirmation and serotyping sent by the network of microbiological laboratories operating in the country and performing primary diagnostics were used. Also data on reported cases of salmonellosis (possible, probable, confirmed) from all 28 Regional Health Administrations (RHAs) on the territory of the country, confirmed by PCR, Rapid-card test or culture methods; weekly and monthly data from the National Centre for Public Health and Analysis (NCPHA) in Bulgaria, as well as annual analyses of communicable diseases in Bulgaria prepared by the Epidemiology Department of the National Centre for Infectious and Parasitic Diseases (NCIPD).

Results. During the study period, a total of 3208 human salmonellosis cases were laboratory confirmed by bacterial culture isolation, of which 1,847 (58%) isolates were from patients with diarrheal syndrome and 1,361 (42%) were isolated from asymptomatic carriers. These bacterial cultures belonged to two species of *Salmonella enterica* and *S. bongori*; 4 subtypes- *enterica*; *salame*; *arizonae* and *diarizonae* and were assigned to 66 *Salmonella* serotypes. The leading aetiological role in patients is represented by *Salmonella Enteritidis*, taking a relative share of 47%, followed by *Salmonella Typhimurium*- 32,8%, of which 40,5%- monophasic *Salmonella* 1,4,5,12: i:- and 2,26% *Salmonella* 1,4,5,12: -: 1,2. *Salmonella* Infantis ranked third among the leading serotypes causing *Salmonella* enteric infections in the country at 4.85%. For the first time in Bulgaria, the following serotypes have been isolated Brazzaville; Larochelle; Ndolo; Kuoka; Jerusalem; Vejle and the unique for the world, new serotype- *Salmonella enterica subsp. enterica* O:11 H: z41 H: enz15. In carriers, in contrast to patients, other serotypes come to the fore-

Muenster; Bazenheid; Dublin, etc. The data obtained from the analysis again confirm that the groups most affected by *Salmonella* infections are infants and young children (<5 years). Reported cases of salmonellosis predominate in the urban population. The sex differentiation between symptomatic patients and asymptomatic *Salmonella* carriers is negligible in favour of the male sex-54%. In 2018, an outbreak of serotype London, a rare serotype in the country, was reported in contrast to the regularly reported outbreaks due to serotype Enteritidis and Typhimurium. An average of 5 *Salmonella* outbreaks have been recorded annually. The results of the retrospective epidemiological study are presented in Tables 1 and table 2, as well as Figures 1, 2, 3, and 4.

Salmonellosis in 2017 - 2023

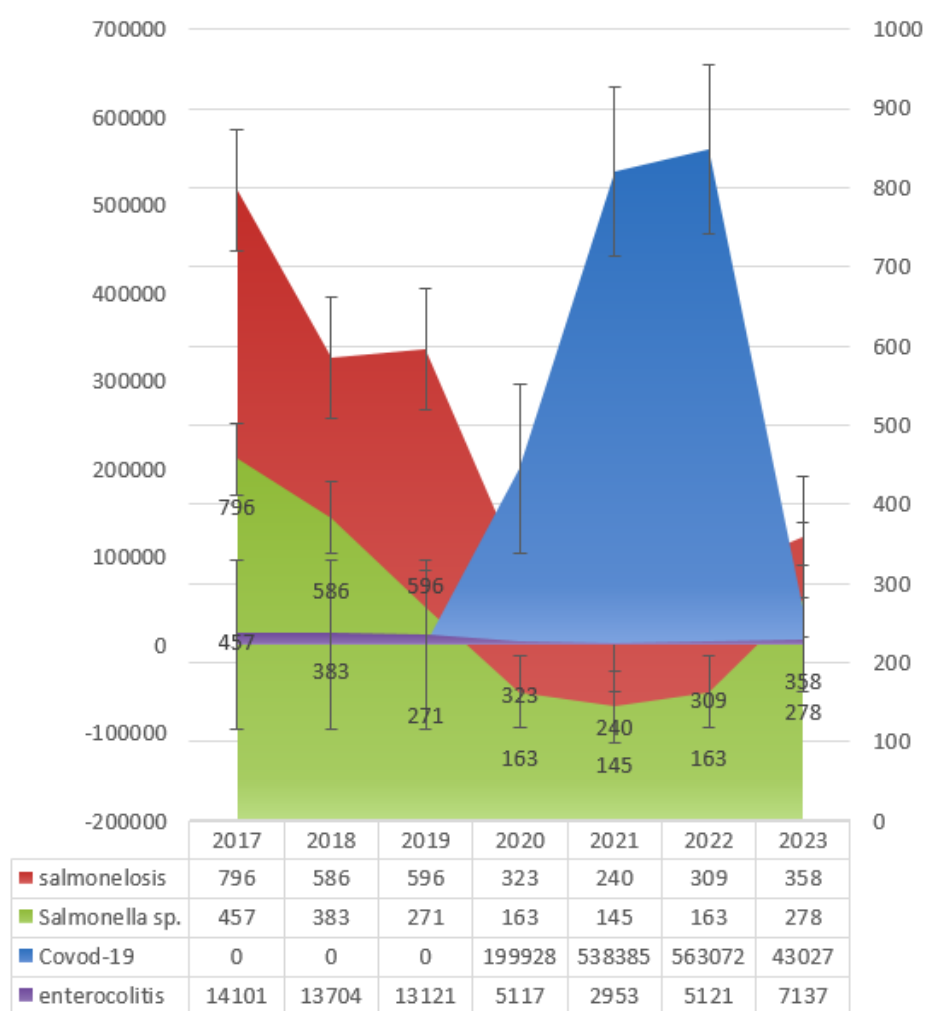


Figure 1. Infectious enterocolitis, reported Salmonellosis and laboratory confirmed human *Salmonella* strains in Bulgaria in the period 2017 - 2023.

Table 1 Attitude of enterocolitis to reported possible/probable Salmonellosis cases and confirmed *Salmonella* serotypes.NCIPD (National Center for Infectious and Parasitic Diseases).NCPHA (National Center of Public Health and Analyses).

year	Enteroclitis	Salmonellosis (NSPHA)	Salmonellosis (NCIPD)
2017	14 101	796	457
2018	13 704	586	388
2019	13 121	596	271
2020	5 117	323	163
2021	2 953	240	145
2022	5 121	309	163
2023	7 137	358	278
общо	61 254	3 208	1 865

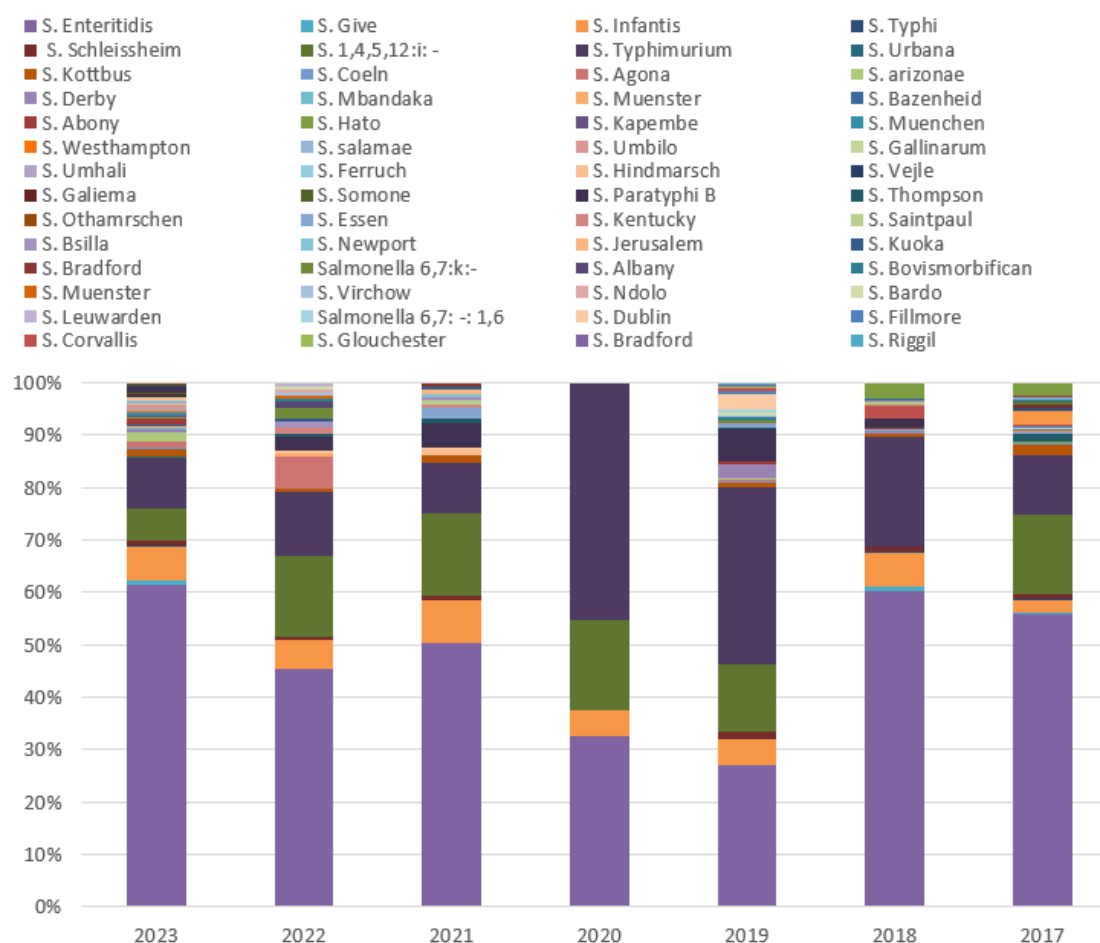


Figure 2 Laboratory confirmed *Salmonella* serotypes in the studied period.

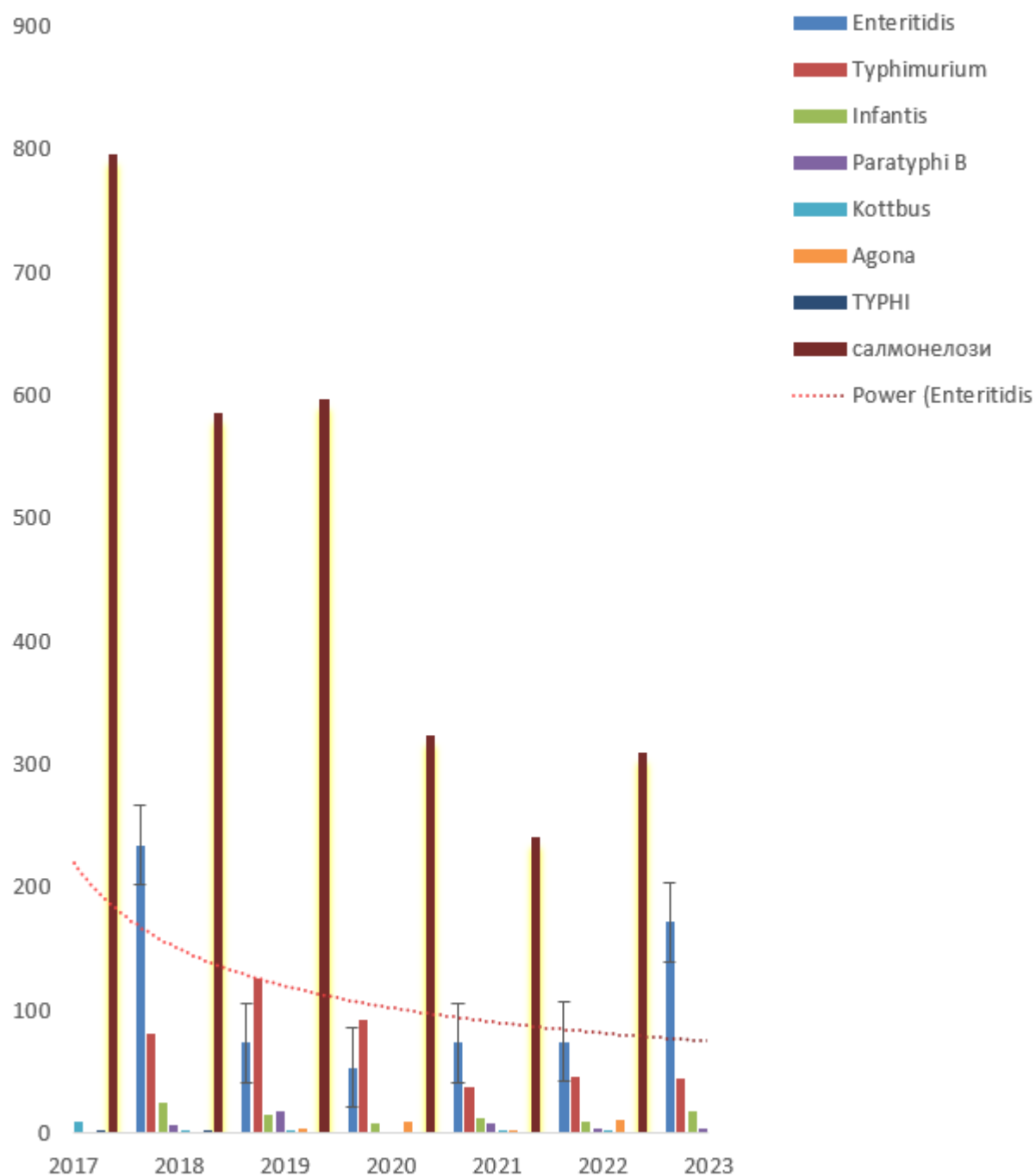


Figure 3 Dynamic and prevalence of *Salmonella* serotypes in Bulgaria for the studied period.

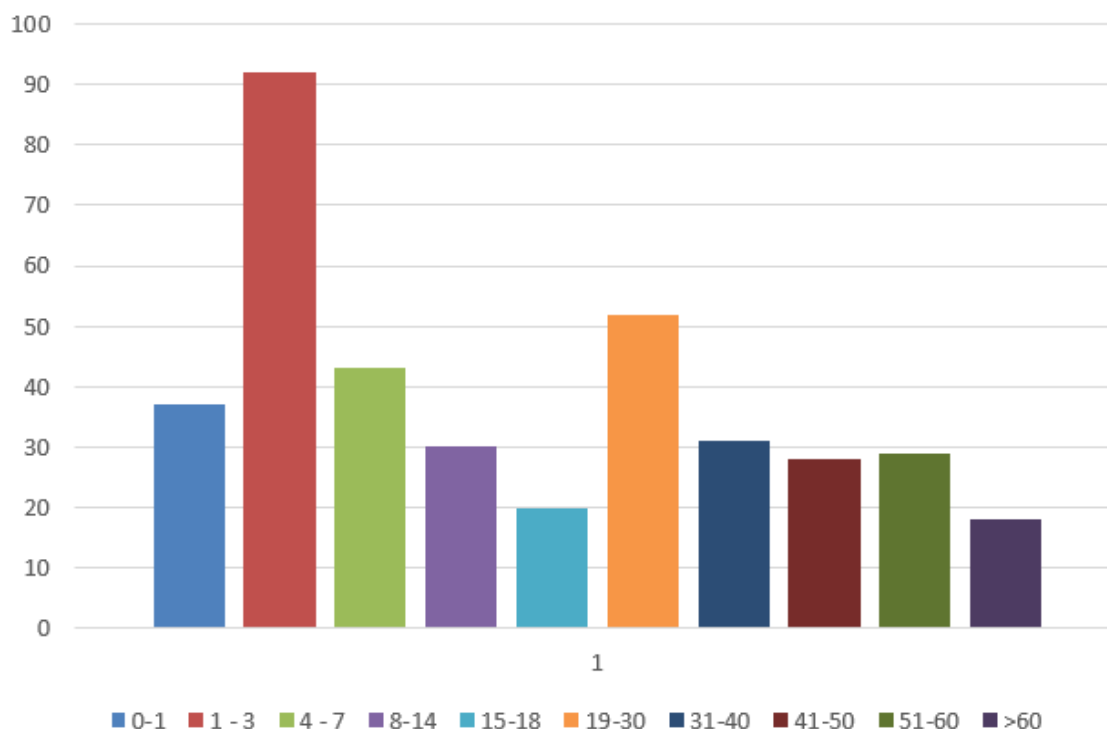


Figure 4 Distribution of *Salmonella* infections by age for 2017-2023 in Bulgaria.

Table 2 Reported multi-country outbreaks caused by *Slamonella* for the period 2017-2023 by ECDC and EFSA. The sources of the outbreaks were food imported into Bulgaria through international marketing. None of the presented outbreaks have been investigated or reported by Bulgaria.

Year	Causative agent	Sources of a <i>Salmonella</i> multi-country outbreak	Outbreak reported by/ Sources imported in	Suspected Reported	References
2017	<i>Salmonella</i> Enteritidis	Hen eggs from Poland	Belgium, the Czech Republic, France, Luxembourg, the Netherlands, Norway, Sweden, the United Kingdom,	Imported in, but NOT reported by Bulgaria	EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2017. Multi-country outbreak of <i>Salmonella</i> Enteritidis infections linked to Polish eggs. EFSA supporting publication 2017: 14(12):EN-1353. 21 pp. doi:10.2903/sp.efsa.2017.EN-1353

			Croatia, Denmark, Finland, Greece, Hungary, Ireland, Italy and Slovenia		
2018	Salmonella Agona	Infant formula from France	Belgium, Bulgaria, Cyprus, the Czech Republic, France, Greece, Ireland, the Netherlands, Romania, Slovenia, Slovakia, Spain and the United Kingdom	Imported, but NOT reported by Bulgaria	<i>EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2018. Multi- country outbreak of Salmonella Agona infections linked to infant formula. EFSA supporting publication 2018: 15(1):EN- 1365. 11 pp. doi: 10.2903/sp.efsa.2018.EN-1365</i>
2021	Salmonella Mbandaka	ready-to-eat chicken products and/or fresh chicken meat from Ukraine	Czechia, Estonia, Finland, France, Germany, Ireland, the Netherlands, the United Kingdom, and Israel	NOT reported by Bulgaria	<i>European Centre for Disease Prevention and Control, European Food Safety Authority, 2024. Multi-country outbreak of Salmonella Mbandaka ST413 linked to consumption of chicken meat products in the EU/EEA and the UK – first update - 21 March 2024.</i>
2021	Salmonella Braenderup	Galia melons from Honduras	Denmark, Austria, Belgium, Czech Republic, Finland, France, Germany, Ireland, Luxembourg, The Netherlands, Norway, Sweden, The UK	Imported in, but NOT reported by Bulgaria	<i>European Centre for Disease Prevention and Control, European Food Safety Authority, 2021. Multi-country outbreak of Salmonella Braenderup ST22, presumed to be linked to imported melons - 20 July 2021. Stockholm: ECDC/EFSA; 2021. https://doi.org/10.2903/sp.efsa.2021.EN-6807</i>
2022	monophasic Salmonella Typhimurium	chocolate products from Belgium	Austria, Belgium, Denmark, France, Germany, Ireland, Italy, Luxembourg, the Netherlands,	Imported in, but NOT reported by Bulgaria	<i>European Centre for Disease Prevention and Control, European Food Safety Authority, 2022. Multi-country outbreak of monophasic Salmonella Typhimurium sequence type 34 infections linked to chocolate products, first update – 18 May 2022.</i>

			Norway, Spain and Sweden, the UK, Switzerland, Canada, and USA		
2023	Salmonella Senftenberg	possibly linked to cherry-like tomatoes from Spain, Morocco, the Netherlands	Austria, Belgium, Czechia, Estonia, Finland, France, Germany, Ireland (1), the Netherlands, Norway, Sweden, the UK, and the US	Suspected for Bulgaria	European Centre for Disease Prevention and Control, European Food Safety Authority, 2023. Multi-country outbreak of Salmonella Senftenberg ST14 infections, possibly linked to cherry-like tomatoes – 27 July 2023.

Discussion. The data confirm studies from our country and other European countries that *S. Enteritidis* and *S. Typhimurium* are the leading causes of non-typhoidal salmonellosis in humans (Figures 2 and 3). As in other EU countries, the number of monophasic *S. Typhimurium* isolates (1,4,5,12: i: -) is increasing year by year at the expense of two-phages *S. Typhimurium* according to ECDC data (ECDC; 2022; Hendriksen, R. S., et. al., 2011). However, unlike the years of the period 1999-2004, where *S. Corvallis* reached statistically significant isolation rates in Bulgaria (Asseva G., et. al., 2006), in the current study period pre and post-COVID-19 pandemic 2017-2023, the serotype *Infantis* significantly displaced *Corvallis* and other common serotypes in the country and in the EU and EEA (ECDC; 2022). Despite the high rate of proven salmonellosis due to *Infantis*, there is no confirmed outbreak, all 97 *S. Infantis* cases are sporadic.

Our results regarding sex and age in the incidence of *Salmonella* in patients with diarrhoeal syndrome are consistent with the literature. The group most vulnerable to these diseases remains the pediatric patient, (Figure 4) in whom the severity of infection depends on host factors and *Salmonella* serotype. *Salmonella* agents usually cause self-limiting gastroenteritis manifested by diarrhoea, abdominal pain, nausea, vomiting and fever, but can sometimes cause serious and potentially life-threatening infection in infants (Zijing Ju, et. al., 2023; WHO, 2023; Wu, L.J., et. al. 2021). An explanation for the high incidence of salmonellosis among young children can be sought in the greater susceptibility to intestinal infections and the physiological characteristics of the child's organism on the one hand, and on the other- in the increased consumption of lyophilized and ready-to-eat foods, from which *Salmonella* are quite often isolated.

S. Muenster, *Bazenheid* and *Dublin* represent the largest relative proportion of *Salmonella* species isolated from asymptomatic carriers. According to the literature, *S. Muenster* is associated with the consumption of milk and milk products (Wood DS, et. al. al., 1984; Radke BR, et. al. al., 2002; van Cauteren D, et. al. al., 2008), *S. Bazenheid* is commonly isolated from chicken (INCE,

SEYYİDE SARIÇAM, et.al.,2022; Gong J,et. al., 2014), while S. Dublin is a well-known serotype causing outbreaks associated with veal consumption (Daniel H. Rice, et. al. al., 1997; Harvey RR, et. al. al. 2017; Paudyal, N., et. al. al., 2019). Again, the finding that these three zoonoses are most commonly isolated from children in the 4-6 years age group is striking (Julie L. Self, et. al., 2023; WHO, 2023). This high prevalence in the child population is likely due to annual mandatory prophylactic examinations for enteric pathogens of children before admission to kindergartens.

In a study assessing the burden of salmonellosis in different European countries, data on the actual incidence of salmonellosis showed that the incidence ranged from 4/100,000 inhabitants in Norway to 2741/100,000 residents in Bulgaria (de Jong, B., et. al., 2006). However, the ways and means of combating these diseases, and above all their prevention, depend primarily on their aetiology and epidemic processes. Bulgaria, as a member of the EU, complies with and applies all directives on the control of communicable diseases and the control and monitoring of food and waterborne infections. The results of the action of this complex of factors are illustrated by the actual assessment of diarrhoeal diseases.

The prevalence of bacterial enterocolitis with *Salmonella* as the aetiological agent in the urban population of Bulgaria is a fact that is contributed by the accessible medical care and the possibility of an aetiology diagnosis of enterocolitis in the cities of the country, where every confirmed case of salmonellosis is reported. However, a realistic picture of the geographic distribution of salmonellosis in the country is lacking, because it is a complex administration task. The reason for this is that the reported laboratory-confirmed cases of salmonellosis are reported to the RHIs, but the samples for testing in most private microbiological laboratories come from different administrative districts of the country. This should be interpreted as a weakness in the communicable disease surveillance system.

According to the available epidemiological data, the new salmonella serotypes in the country are mostly associated with cross-border travel outside the EU: Egypt, Philippines, Zanzibar, Bali, India, Thailand, Turkey, Maldives, and according to the patients, the manifestation of diarrhoea started during their journey. Globalisation, affordable transportation and the import and export of goods contribute to both the socioeconomic boom and the widespread transmission of pathogens all over the world (Edoardo Pozio, 2020)

In the years of the Covid-19 pandemic, the diagnosis of bacterial enteric pathogens was neglected, leading to an increase in the number of “hidden infections, followed by an increase in non-aetiologically, inadequate antimicrobial therapy, which contributed to the spread of new antibiotic resistance profiles and a potential increase in the incidences of infections in Bulgaria (Figure 1). The impact of the respiratory pandemic has similarly affected all EU countries, according to the ECDC (ECDC; 2022). Figure 1 and Table 1 show a mirrored increase in 2023 of reported *Salmonella* cases, reaching their 2019 levels. This trend is a clear sign of an increase in Salmonella cases soon.

It is evident from the foregoing that salmonella infections are a complex problem, which makes it necessary to know and monitor their etiological structure in the struggle for their containment. However, reported outbreaks in Bulgaria are decreasing year by year. This fact is due, on the one

hand, to the population's neglect and disregard of the diarrhoeal syndrome, failure to seek medical help in time, and the widespread use of differential microbiological diagnostics limited to bacterial species, to the *Salmonella* group and, increasingly rarely, to a limited number of serotypes. All multi-country *Salmonella* outbreaks presented in a table 2 have not been identified, nor reported within the country, despite evidence of imports of food and animal products identified as sources of the described outbreaks.

Conclusion. Enteric infections caused by *Salmonella* continue to be of socio-economic importance. Salmonellosis is a challenging public health problem that requires a good understanding of its aetiological structure and dynamic surveillance in the fight to reduce it.

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